# Fish Analgesia: Pain, Stress, Fear Aversion, or Nociception?

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**KEYWORDS**

- Fish • Stress • Analgesia • Neopallium • Nociception

Fish represent the largest class of vertebrate animals with more than 31,000 known species. The author estimates over 3000 species of these animals are used for a variety of purposes such as a protein source for humans and other animals, recreational and commercial fishing, companion animals, display animals, and research. To meet the ever increasing demands placed on fish, aquatic animal medicine is growing to help support veterinary needs for aquaculture, companion animal medicine, conservation medicine, laboratory animal medicine, zoo and aquarium medicine, ecosystem health, and wild fisheries for sport, recreation, restocking, and protection of endangered species. The increasing use of fish resources and a greater understanding of aquatic animal medicine demands providing evidence-based veterinary care for these animals. Because fish are aquatic as well as being pokilothermic, there are several unique anatomic and physiologic considerations that must be understood when working with these animals. Veterinarians need to adapt methodologies for examining, performing diagnostics, and treating fish patients to decrease stress, decrease fear, and avoid and/or decrease nociception. Recently several works have been published on the topic of fish anesthesia and analgesia in laboratory animal medicine, and these contributions are highly recommended for veterinarians seeking to get more information on these topics.[1,2] This article briefly defines stress, reviews and compares fish neuroanatomic pathways associated with nociception, discusses behavioral observations, summarizes current use of analgesics for fish patients, and concludes with the ongoing controversy regarding pain on this topic. With increasing concern on animal welfare in Europe and North America, veterinarians will also have a vital role contributing to objective research for better understanding the short- and long-term consequences of noxious stimuli as related to health and well-being for fish, reptiles, amphibians, and invertebrates.[3,4]

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The author has nothing to disclose.

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Vet Clin Exot Anim 14 (2011) 21–32
doi:10.1016/j.cvex.2010.09.002 **vetexotic.theclinics.com**
1094-9194/11/$ – see front matter © 2011 Elsevier Inc. All rights reserved.

## STRESS IN FISH

Although there is more and more research being conducted using fish, stress is poorly defined for this and other classes of pokilothermic vertebrate and invertebrate animals. Homeostasis for fish patients refers to overall biologic and physiologic balance, or to the well-being of the host in its environmental conditions. Veterinarians try to maintain this balance and/or attempt to correct imbalances when presented with a fish patient. Barton and Iwama[5] defined stress as any event or action that challenged the homeostasis of the nearly resting animal. There are numerous studies, and it is well accepted that fish respond to stress with physiologic similarities and differences to other higher and lower vertebrates. Stress for fish patients involves a complicated integration of the animal and its environment. These stressors can serve to threaten or disturb the animal's homeostasis and cause a cascade of physiologic responses meant to be adaptive or compensatory.[6]

Overall disruption of normal physiology can lead to disease and is dependent on the individual's health, as well as intensity and duration of the stress or stressors. Fish disease can be most simply defined as an overall failure of the animal or animals to thrive. Some of the more common stressors identified for fish patients include: changes in either chemical or physical water quality conditions (water temperature, pH, alkalinity, salinity, DO); accumulation of nitrogenous waste in water; other environmental pollution; handling; transport; excess noise; poor/inadequate nutrition; overstocking; aggression; predation; infectious disease (parasitic, bacterial, viral, fungal); and many others. These stressors can invoke primary, secondary, and tertiary responses.[5] The fish neuroendocrine pathways for stress are very similar to other vertebrates, and they rely on the adrenergic system and the hypothalamic pituitary-interrenal (HPI) axis. The HPI axis is activated in response to stressors or noxious stimuli, and begins a central mediated cascade of events triggered by corticotropin-releasing factor (CRF) in all vertebrates studied to date.[7] When fish respond to stress acting on primary receptors or through a nerve-mediated motor response, a primary cascade begins to elicit an alarm response for fight or flight, with stimulation of the central nervous system (CNS) causing the hypothalamus and pituitary to release adrenocorticotropic hormone (ACTH).[8,9] Sympathetic neurons also send impulses to the teleost analogue for adrenal tissue that are called chromaffin cells. Chromaffin cells are grouped in clusters and scattered throughout the posterior kidney and posterior cardinal vein to release catecholamines such as epinephrine and norepinephrine. Simultaneously, the HPI axis causes release of corticosteroids from interrenal tissue also located within the posterior kidney, which are distributed throughout the bloodstream and cause physiologic changes, allowing the animal to respond.[8–11]

The consequence of the primary response can lead to secondary responses for resistance and adaptation if the stress duration persists, as many of the steroids can have longer-term effects than the initial catecholamine release. These secondary responses, as a result of circulating cortisol and catecholamines, can result in increased cardiac output, metabolic rate, respiration, plasma free fatty acids, lactic acidosis, and hematocrit, and have a varying effect on liver glycolysis and electrolyte balance.[12–16]

Tertiary responses are the result of chronic stress leading to physiologic exhaustion and can have long-term consequences, negatively affecting teleost immune function, locomotion/swimming, metabolism, reproduction, growth rate, behavior, and overall survival.[5,12,17–19]

By understanding the general stress response in fish, veterinarians will be able to assess many nonspecific signs of fish in distress marked by behavioral changes, anorexia, swimming, increased respiratory rate, increased heart rate, decreased body condition/condition factor, and changes in complete blood counts, hematocrit, glucose, and electrolyte values. Given the consequences of tertiary stress on fish patients, every effort should be made to manage and alleviate stressors in clinically ill patients when handling, diagnosing, treating, and maintaining fish patients for treatment, hospitalization, and quarantine. To ameliorate stress from the general examination through subsequent hospitalization for the numerous species that may be encountered, veterinarians should use practical husbandry solutions specific to the species, which may include decreasing water temperature, adding 1 to 3 ppt of sodium chloride to freshwater fishes, decreasing salinity for marine fish by 3–5 ppt, fasting before transport or procedures, reducing stocking densities, decreasing anesthetic time, and/or providing hide boxes or artificial plant material.[16,20] Not all anesthetic agents, sedatives, and analgesics used in fish medicine may have an effect on the stress cascade as described. Some agents may cause corticosteriod or catecholamine release when used, such as observed in Atlantic salmon (*Salmo salar*), Atlantic cod (*Gadus morhua*), and Atlantic halibut (*Hippoglossus hippoglossus*) with benzocaine, MS-222, metomidate, and isoeugenol.[21]

## FISH NEUROANATOMY

Neuroanatomy is largely conserved among vertebrate animals within the peripheral nervous system and CNS.[22] A comprehensive discussion of fish neuroanatomy can be found in other publications.[23–25] A great deal more research needs to be completed, given the diversity of species in this class of vertebrates.

The peripheral nervous system consists of somatic and autonomic divisions, with the former responsible for motor skills and the latter being further subdivided into the sympathetic, parasympathetic, and enteric. The somatic nervous system controls voluntary body movements and maintains the body in its surroundings based on input from the various sensory systems. Divisions of the autonomic nervous system include the sympathetic nervous system, to which most often is attributed the “fight or flight” response that controls the response to stressful stimuli, and the parasympathetic nervous system that tries to be an antagonist to this response, conserving energy.

The peripheral nervous system is responsible for transmitting stimuli detected by nociceptors to the CNS. Nociceptors are bare axon nerve endings that are modified and specialized to detect temperature, pressure, and/or chemoreception terminating at the spinal nerves. Two types of polymodal fibers have been described in fish, namely A-delta fibers (Aδ fibers) and C fibers.[26] Aδ fibers are thin, myelinated, and can conduct information at moderate to fast speeds. These nerve fibers are afferent, detecting noxious stimuli and evoking immediate withdrawal from stimuli. By contrast, C fibers are unmyelinated and have slow conduction velocity, but respond to a stronger intensity of stimulus and are responsible for the dull, peracute and chronic, pain. Polymodal C fibers can adapt to a variety of physiologic changes with responses to hypoxia, hypercapnea, hyperthermia, hypothermia, hypoglycemia, hyperosmolarity, hypoosmolarity, and lactic acidosis.[27] Of the 3 major classes of fish, Osteichthyes (bony fishes) have both types of fibers although it is reported that Aδ fibers are a predominant type[28,29]; only Aδ fibers have been identified in Chrondrichthyes (elasmobranchs),[30] whereas the Agnatha (hagfishes and lamprey) primarily have C-type fibers.[31]

Nociceptive signals are transmitted from the nociceptors through the peripheral nerves, and are relayed via nerves of the spinothalamic and trigeminal tract in similar fashion to other vertebrates.[29,32] These nerves then relay information to the brain for processing.

The CNS of fish contains a spinal cord, medulla oblongata, and the brain, divided into telencephalon, diencephalon, mesencephalon, and cerebellum. Nociceptive processing occurs primarily in the forebrain that includes the diencephalon and telencephalon, with the former divided into epithalamus, thalamus, and hypothalamus, and the latter cerebral hemispheres. In fish, similar to other vertebrates, nociception travels from the peripheral nerves via the spinal nerves, and is relayed through the spinal cord to the thalamus. The thalamus is connected to the telencephalon via multiple connections through the gray matter pallium, which has been demonstrated to receive nerve relays for noxious and mechanical stimilu.[33] Some notable differences in the fish telencephalon are a thin roof plate and general lack of ventricle and ventricular sulcus.[23] Although there are differences in brain structure, it has been demonstrated that certain species of fish are able to process nociceptive responses in the brain, as demonstrated in comparisons between goldfish (*Carassius auratus*) and rainbow trout (*Oncorhynchus mykiss*) showing both species had neuronal activity in the spinal cord, cerebellum, tectum, and telencephalon from mechanoreceptive and nociceptive stimuli; and further demonstrating that all fish are not the same, goldfish having exhibited greater neuronal activity than rainbow trout when receiving noxious versus mechanical stimuli.[33]

In endothermic vertebrates the general pain pathway involves complex interactions of peripheral nerves, spinal cord, and brain. These interactions begin with nociceptor nerve endings modified and specialized to detect temperature, pressure, and/or chemoreception, which are bare-axoned and terminate in the spinal cord. A painful stimulus detected by the nociceptors travels via electrical impulse through the spinal cord where other specialized neurons serve to relay, ignore, or amplify the response to the brain. These impulses then travel to the thalamus, and are sent to the cerebral cortex and neocortex or limbic center. These 2 areas then elicit response consisting of a coordinated motor response from the cerebral cortex and an emotional response from the neocortex. This emotional response is what is largely accepted as allowing conscious thought and ultimately pain.[26,27] The 6-layer neocortex or neopallium is the outer layer of the cerebral hemispheres, and 1 of the 3 components of the cerebral cortex (neocortex, archicortex, and paleocortex). Reptiles were thought to have first evolved the neocortex in vertebrates, but the neopallium is absent in amphibians and fish.[34] In fish the archipallium is the largest part of the cerebrum, and is believed to have developed into the hippocampus in other vertebrates.[35] These differences in brain function between classes of vertebrates, in addition to greater variation among fish species, complicate functional and physiologic comparisons with inference to the human concept of pain. Because fish and amphibians lack a neopallium, this difference is the foundation for debate among researchers over whether these animals feel pain, and is discussed briefly in this article.

### *Neurotransmitters*

The physical networks of nerves are the highway for these connections to travel from the periphery and be processed centrally, but they are heavily dependent on both local and systemic effects of several biochemical neurotransmitters or neuromodulators. These substances include endogenous opioid compounds, opiate-related compounds, substance P, and Mauthner cells, and are produced in various anatomic locations, and act on receptors in the brain and spinal cord. Substance P is a neuropeptide with receptors found throughout the CNS. Substance P and other sensory neuropeptides may be involved with neurogenic inflammation and are released from peripheral nerve fibers at the site of noxious stimuli in response to injury or infection.[36] In 1956, substance P was discovered in the intestine and the brain of fish and was shown to have depressive, hyperalgesic, and morphine antagonist effects on the CNS.[37,38] Evidence for endogenous opioids and opioid receptors began with discovery of β-endorphin–like immunoreactive substance in the brain of *Boops boops* and understanding that these compounds are involved with a hypophysial regulatory factor or neuromodulatory agent, similar to other vertebrates.[39] All 4 main opioid receptor types (delta, kappa, mu, and NOP) were found to be conserved in vertebrates, even in primitive jawless fishes (agnathastoma).[40] In addition, endogenous opioids were noted to affect serum gonadotropic hormone (GtH) levels in male goldfish, and were identified during reproduction for male brown bullhead catfish, *Ictalurus nebulosus* Lesueur.[41,42] The endogenous opioid peptide system in male brown bullhead catfish is characterized by naloxone binding and the response to naloxone during the annual reproductive cycle. More direct evidence for the presence of endogenous opioid peptides was revealed using immunohistochemistry to locate α-melanotrophin in the pars intermedia of the Australian lungfish *Neoceratodus forsteri*, and met-enkephalin in the CNS of teleosts *Anguilla rostrata* and *Oncorhynchus kisutch*.[43,44] The $GABA_A$ receptor ionically binds γ-aminobutyric acid (GABA) to inhibit neurotransmission in the CNS. The effects mediated by this ligand-gated ion channel make it a target for benzodiazepine (BZ) tranquilizers. These BZ binding sites have also been identified and partially characterized in whole brain membranes of male rainbow trout.[45] Mauthner cells are special cells in teleost fish, believed to initiate escape responses.[46] The presence or absence of Mauthner cells shows a clear correlation and dramatic differences in fast-start behavior when compared in several species of puffer fish.[47]

## OBJECTIVE AND SUBJECTIVE BEHAVIORAL EVIDENCE OF PAIN IN FISH

Although some of the evidence for whether fish feel pain is found in neuroanatomy and neurophysiology, behavior is commonly used to discern pain in other animals and human infants. When monitoring fish patients for clinical signs of distress, some considerations include the subjective assessments of body orientation and swimming, feeding, hiding, and/or spatial positioning (near aeration or heater). Additional parameters are more medically specific and objective, for example, environmental quality parameters, heart rate, opercular or respiratory rate, lethargy, fin clamping, darkening, diagnostic values (eg, complete blood count and blood chemistries), and/or physical examination findings.

When examining a fish patient, vital signs are as critical as for mammalian patients. It has been well demonstrated that increased respiratory rate and cardiac output can be observed when brown trout (*Salmo trutta*) are subjected to either handling stress[48] or rainbow trout to noxious stimuli simulated by bee venom or acetic acid solution into the lip.[49]

Much of the controversy about identifying stress, pain, and/or distress in fish surrounds a lack of critical and comprehensive clinical data for a majority of species. Noticing changes in diagnostic results for fish is challenging, as normal baselines for many species are not readily available. When abnormalities are detected it is important to place this in the context of the entire physical examination, environmental quality parameters, and observation for a potentially ill fish patient. Several changes in blood chemistry have been observed in largemouth bass (*Micropterus salmoides*) from tournament-caught fish in a catch-and-release tournament that were sampled within 5 minutes after official tournament weigh-in.[50] Several chemistry parameters for these animals were significantly greater than controls, including plasma cortisol, glucose, sodium, and potassium, and large changes in metabolic status for these fish included major reductions in the muscle energy stores phosphocreatine, adenosine triphosphate, and glycogen, and large increases in muscle and plasma lactate concentrations.[50]

Fish can exhibit abnormal behavior when ill, and a variety of these behaviors have been documented. Rainbow trout treated with noxious stimuli injected into their lips can also have unusual behavior such as rocking on either pectoral fin from side to side, and also rubbing their lips into the gravel and against the sides of the tank.[49]

One of the most common subjective reasons for providing pain medication in fish patients is to eliminate anorexia. Inappetence or anorexia is a common finding of compromised fish patients, and can be a complication after anesthesia, after diagnostic procedures, and/or following surgery. There is greater evidence for the stress mechanisms in causing fish to become anorectic. In rainbow trout chronic plasma cortisol elevation affected individual appetite with subsequent decreases in growth rate, condition factor, and food conversion efficiency.[51] Using CNS injections of CRF and the related peptide, urotensin I (UI) in goldfish, appetite suppression occurred in a dose-related manner, and after intracoelomic CRF receptor antagonists were implanted in fish as glucocorticoid receptor antagonists, the blocked receptors reversed the appetite-suppressing effects previously observed.[52] Ammonia is toxic to fish, and many species exhibit anorexia when exposed to high ammonia levels. Investigators using rainbow trout to understand the appetite-suppressing effects of high environmental ammonia analyzed food intake after exposing fish to various doses of NH3, and have noted that appetite decreased in a dose-dependent manner after 24 h of ammonia exposure.[53] In these animals brain serotonin (5-HT) was the key neuromodulator associated with this change of anorectic behavior due to high ammonia.[53]

There are also numerous researchers dedicating time to understanding avoidance behavior exhibited by fish, which is relevant to the current debate and discussion. An example of behavioral responses to acute noxious stimuli comparing rainbow trout with goldfish involved observing animals after administering electric shocks of 2 different intensities directly to the skin; goldfish demonstrated spatially cued shock avoidance with significantly improved shock-avoidance learning, whereas trout displayed a difference when a conspecific was presented and remained in the area of the conspecific when it was being subjected to the stimuli.[54]

## ANALGESIA IN FISH

In brief, anesthesia and/or sedation can be administered via bath, orally, or via injection using a variety of substances that have been used to anesthetize fish including ethanol, diethylether, benzodiazepines, halothane, lidocaine, benzocaine, tricaine methanesulfonate, eugenol, ketamine, medetomidine, propofol, carbon dioxide, iso-eugenol, oxygen, and many other substances.[55] In the United States there is one federally approved anesthetic agent, tricaine methanesulfonate, also called MS222, which is added to water as a bath application. Tricaine methanesulfonate is used for a variety of species, and many teleost and elasmobranch fish can be induced at a dose of 75 to 110 mg/L.[55] The reader is referred to a recent review by Ross and Ross[56] on anesthetic and sedative techniques for aquatic animals. It should be noted, as previously stated in this article, that anesthetic agents in fish may not always provide analgesic effects and can increase or have no effect on the stress response of fish.

Nociception is the response of an animal to a noxious stimulus, and is a current topic in research circles and for animal welfare concerns related to fish.[57–63] Research pharmacology that provides analgesic drugs and dosages for fish patients is scarce in veterinary literature; however, most veterinarians assume that fish behave negatively to noxious stimulus, and use analgesics to ameliorate the nociceptive response or pain perceived in these animals. Comparisons have been made using intraoperative administration of the opiate butorphanol (0.4 mg/kg) or the nonsteroidal anti-inflammatory analgesic ketoprofen (2 mg/kg) in koi (*Cyprinus carpio*).[64] In this study the untreated controls and ketoprofen-treated fish had decreased activity and appetite after surgery, in contrast to the butorphanol-treated group; in addition, decreased creatinine kinase was noted in the ketoprofen group, suggesting a mild behavioral sparing effect of butorphanol and reduced muscle damage from the ketoprofen.[64] Using morphine, winter flounder (*Pseudopleuronectes americanus*) given a noxious stimulus such as the injection of acetic acid resulted without an acute change in cardiac response, but subsequent long-term increases of heart rate and cardiac output were noted.[65] When morphine was administered to rainbow trout that were injected with a noxious substance in the lip, these animals exhibited less abnormal behavior and had a decreased respiratory rate compared with untreated controls.[66] Similarly, when rainbow trout were given an opiate antagonist after morphine, administration of electrical impulses to produce agitated swimming responses resulted in significantly reduced analgesic effects compared with controls, in a dose-dependent fashion from 20 to 150 minutes.[67]

In common carp (*Cyprinus carpio*) a prolonged analgesic response was observed when tramadol, an agonist of the opioid mu receptor, was given before applying a noxious electrical stimulus. The effect was observed to be both dose dependent and to last for 2 hours, with fish maintaining normal swimming and their normal behavioral repertoire compared with controls that did not receive tramadol.[68] Unlike teleosts, elasmobranchs such as chain dogfish (*Scyliorhinus retifer*), when administered multiple doses of each of the analgesics (0.25, 0.5, 1.0, 2.5, and 5.0 mg/kg butorphanol and 1.0, 1.5, 2.0, and 4.0 mg/kg ketoprofen) before undergoing anesthesia did not have a significant reduction of anesthetic concentration with MS-222.[69]

Many nonsteroidal anti-inflammatory drugs (NSAIDs) and opiates are available to veterinarians, such as carprofen, meloxicam, ketoprofen, flunixin meglumine, butorphanol, tramadol, morphine, and buprenorphine.[55] Because pharmacokinetic studies are not available for many of these compounds for the variety of fish species commonly seen as patients, empirical dosing may be used. The most common application for analgesia in fish is for surgery, trauma, cutaneous ulceration, or inflammatory lesions.[70] Some drug doses commonly used are shown in **Table 1**. No analgesics are currently approved in the United States for use in fish and these dosages are limited to several ornamental companion animal species, so they should be used cautiously and never in fish intended for human consumption.

## PAIN IN FISH

The question of whether fish feel pain is extremely and pointedly relevant as veterinary expertise continues to offer a greater range of nonlethal diagnostic and treatment options for fish patients. It is a question posed by clients as well as the general public for a variety of reasons stemming from fish surgery to catch-and-release sport fishing, and from fish research to the evolving views on animal welfare. Whether fish feel pain is also increasingly debated among researchers, with the most publicized exchange on this issue being documented in the scientific literature between Drs Sneddon[49,58,66] and Rose.[59,63] These debates largely stem from the evolution and anatomy of the fish brain, which lacks a neopallium, the site where pain is thought to be perceived in higher homoeothermic vertebrates, as compared with other research on nociception, behavior, and opioid receptors and endogenous opioids conserved in fish.[71]

**Table 1**
**Fish analgesic agents and dosages: commonly used analgesics in veterinary medicine that have been used in fish patients**

| Drug | Dose (mg/kg) | Route | Interval (hours) | References | Comments |
|---|---|---|---|---|---|
| Opioids | | | | | |
| Buprenorphine | Not recorded: 0.01–0.02 in cats and dogs | | | [55] | Wide range of safety and efficacy |
| Butorphanol | 0.1–0.4 | IM | 24 | [55,64] | Dose varies species to species with efficacy |
| Morphine | 0.3 | IM | | [70] | Used in research; dose varies species to species |
| Oxymorphone | Not recorded | | | | 10× potency of morphine |
| Tramadol | 5.0–10.0 | PO | 48–72 | [68] | |
| NSAIDs and others | | | | | |
| Carprofen | 2.0–4.0 | IM | 72–96 | [70] | NSAID side effects; gastric ulceration observed in sand tiger shark (*Carcharias taurus*) with long-term use |
| Flunixin | 0.25–0.5 | IM | 72–96 | [55] | |
| Ketoprofen | 2.0 | IM | | [64,69] | |
| Meloxicam | 0.1–0.2 | IM | 24–48 | [70] | |

*Abbreviations:* IM, intramuscular; NSAID, nonsteroidal anti-inflammatory drug; PO, by mouth.

Whether fish feel pain, are simply fear aversive, or are experiencing nociception to any noxious stimulus caused from disease, poor husbandry, handling, or transport, it is a veterinary responsibility to help ameliorate this stress and/or distress in a similar way to practical veterinary solutions employed for other livestock, pet, laboratory animal, and wild populations.

## ACKNOWLEDGMENTS

I want to dedicate this article to all my fish patients over the years that have allowed me to evolve and grow in my knowledge of aquatic animal medicine, and also to Dr Greg Lewbart, who has mentored me over the years and first inspired me to pursue my veterinary goals in aquatic animal health.

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